

AGRAULIS AND PASSIFLORA I. CONTROL OF SPECIFICITY¹

NEWTON H. COPP² AND DEMOREST DAVENPORT

*Department of Biological Sciences, University of California,
Santa Barbara, California 93106*

Butterflies in the tribe Heliconiini (Nymphalidae) exhibit a highly specific association with one family of dicotyledonous plants, the Passifloraceae (passion-vines). Research on heliconians and passion-vines at the community level suggests that many aspects of the butterflies' sophisticated behavioral capabilities and sensory systems have evolved in the context of the exploitation of larval food-plant resources, and that the passion-vines have responded in turn with a diverse array of morphological "defenses" against heliconian predation (Benson, Brown, and Gilbert, 1975; Gilbert, 1975). This constitutes an extension of Ehrlich and Raven's (1964) proposed model for the coevolution of butterfly herbivores and their plant hosts, which considered only the stepwise proliferation by the plant and subsequent circumvention by an insect of chemical barriers to herbivory.

In spite of extensive observations on the natural history and general behavior of heliconians (Crane, 1955, 1957; Alexander, 1961a, b), information concerning the sensory modalities and behavior involved in the recognition of specific host-plants by heliconians remains to a great extent anecdotal. No experimental investigation into the methods of host-plant selection by a single species of heliconian has been undertaken to our knowledge. Such information is critical in testing postulated cause-effect relationships between heliconian behavior and *Passiflora* morphology.

This problem was approached in regard to the heliconian *Agraulis vanillae* and its passion-vine host plants. In the neotropics, *Agraulis* preferentially infests vines in the *Passiflora* subgenus *Granadilla* (Benson et al., 1975).

The following experiments examine the role of butterfly behavior and host-specific mortality in determining the infestation levels of various species of *Passiflora* by *Agraulis* in Santa Barbara, California.

MATERIALS AND METHODS

The butterflies

Laboratory cultures of *Agraulis vanillae incarnata* (Riley), started in the spring of 1973 from wild stock collected locally in Santa Barbara, California, were maintained in a controlled-environment room on a 15L:9D cycle at 23° C and 60% humidity. Adults were placed in nylon screened cages 61 cm square and 91 cm high. In these cages they fed either on 1 M sucrose in sponges or on a 20% honey solution in artificial red flowers which was changed every other

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² Current address: Department of Biology, University of Redlands, Redlands, California 92373.

day. A small piece of red tape in the center of the sponges increased their attractiveness to the adults.

In addition to the overhead Cool-White fluorescent room lights, each cage was illuminated by one 150W-120V GE reflector flood, suspended 25 cm above the top of the cage. These lights cycled on a 13L:11D regime coordinated with the room lights to provide one hour of "dawn" and one hour of "twilight." Under these conditions the average adult life span was 10-16 days. The adults adjusted well to caged conditions. Apparently, mating and oviposition proceeded normally. Females oviposited on fresh new-growth shoots of *Passiflora*. Eggs on these shoots were then transferred to wire screen cylinders (19.5 cm in diameter, 45 cm high) closed at the top and bottom by the cover and bowl of a plastic cake pan. Fresh vine of the species on which the larvae had hatched was provided as necessary until pupation. Shortly after emergence the adults were transferred from these wire cylinders to the screen cages.

Females laid very few eggs on two species of *Passiflora*: *P. mollissima* and *P. edulis*. Larval cultures on these two species were established by transferring newly hatched larvae from the screen of the adult cages (where females frequently oviposited) to fresh new-growth shoots of these vines.

Once a year, in the summer, butterflies from the local wild population were added to the laboratory cultures to maintain a high frequency of wild-type genes.

The plants

Six species of *Passiflora*, each locally available in Santa Barbara County, were used in the investigation: *P. alato-caculca*, hereinafter referred to as AC, (the familiar garden hybrid, *P. alata* \times *P. caculca*, both parental types in the subgenus *Granadilla*); *P. caculca* (referred to as CR); *P. manicata* (referred to as MN; subgenus *Granadillastrum*); *P. mollissima* (referred to as ML; subgenus *Tacsonia*); *P. edulis* (referred to as ED; subgenus *Granadilla*); and a hybrid of unknown origin, *Passiflora* sp. (referred to as SP), which is morphologically intermediate between *P. manicata* and *P. mollissima* (Killip, 1938). None of these is native to California, although *P. manicata* may occur as a garden escape. All specimens were located in the gardens of private residences. For any one species the same specimen of vine provided material for all experiments and cultures. Occasionally alternate sources for a vine species replaced the standard source if it became unavailable.

The species of vine which serves as the larval food plant for an experimental sample of butterflies is hereinafter referred to as the insects' "host" species. Butterfly lineages on the different species of *Passiflora* were not kept pure except where specified. Eggs used to establish one culture were generally contributed by adults from several other cultures. This mixing of lineages helped to prevent the formation of separate strains of butterflies each adapted to a different host-plant species.

Larval food choice experiments

The larvae of many temperate zone lepidopterans exhibit feeding preferences which vary according to feeding experience in earlier instars (Jerny, Hanson,

and Dethier, 1968; Wiklund, 1973; Yamamoto, 1974; Städler and Hanson, University of Maryland, personal communication). Such preferences may affect host-plant selection either directly as a result of the larvae moving from one species of plant to another (Hanson, University of Maryland, personal communication) or indirectly by affecting the ovipositional preferences of subsequent adult females as stated in Hopkins' host selection principle (Hopkins, 1917; Craighead, 1921). The induction of feeding preferences in the larvae of *Agraulis* was tested for in the following manner.

Fourth-instar larvae, raised exclusively on one species of *Passiflora*, were tested in single choice situations matching agar plates containing the mashed leaves of one species of *Passiflora* against agar plates containing mashed leaves of another species. This technique necessarily eliminates any influence of nonchemical factors such as leaf surface texture on feeding specificity; however, all experiments known to us indicate that chemical agents primarily determine the specificity of feeding responses of larval lepidopterans and most other oligophagous insects (*c.g.*, Dethier, 1966; Schoonhoven, 1968).

The plates containing ground leaves of *Passiflora* were prepared as follows: 7 grams of Bactoagar were dissolved in 210 ml boiling water and the solution cooled to 68° C. Eight grams of fresh leaves, taken from new-growth shoots about 18 inches long, were ground in 100 ml water in a blender for 30 seconds. The cooled agar solution was added to this unfiltered leaf mash and blended for an additional 15 seconds. "Unlabeled" agar plates contained exactly the same formula without leaf mash.

The warm agar-leaf mixture was poured in a 3 mm thick layer into two diagonally opposed quadrants of a 25 cm diameter glass finger bowl. The remaining two quadrants received either an unlabeled agar solution or one containing leaf mash of a different species of *Passiflora*. The agar surfaces in adjacent quadrants abutted against each other and, when all were labeled with leaf mash, showed little discernable difference in color or texture. A plastic ring 7 mm high was inserted in a 5 cm diameter circle cleared of agar in the center of the bowl where all four quadrants met. After being placed in this ring larvae tended to mount it and walk some distance around it before moving onto the agar, thus increasing the probability of larval contact with both types of label before feeding.

A single experimental trial consisted of placing five fourth-instar larvae in the ring of an experimental dish and counting the number of fecal pellets deposited within each quadrant after 24 hours of feeding. The larvae had been removed from their host-plant four hours prior to an experiment, during which time they voided residual frass. This ensured that a count of the fecal pellets left in each quadrant of the experimental dishes at the end of 24 hours gave a quantitative measure of the feeding that had taken place on that quadrant during the experiment. Ten experimental trials (*i.e.*, fifty larvae) comprised each choice situation.

Food choice experiments for a single culture consisted of pairing the host-plant species of that culture with each of the other five species. All experiments were conducted in the culture laboratory (23° C, 60% humidity). Control tests, in which a total of fifty larvae (*i.e.* ten experimental trials) faced a feeding

choice between quadrants labeled with mashed leaves of CR and unlabelled quadrants, were carried out either in the dark or with green paper taped to the underside of the bowls to control for visual cues.

Adult ovipositional choice experiments

Female specimens of *Agraulis*, raised as larvae on only one species of *Passiflora*, oviposited in single choice experiments which matched the female's larval food plant with one of the other five species. Choice situations consisted of placing four new-growth shoots of *Passiflora*, two from the host species and two from one of the other five, in 50 ml flasks of water and arranging them alternately in the back of a cage. Adjusting the number of leaves on each 18-inch sprig controlled for differences in leaf-surface area. The sprigs remained in place for 24 hours, were then reversed in order and left an additional 24 hours. The number of eggs on each species of vine was then counted. Since the females fly for a time after the deposition of each egg, significant differences in total egg count between two species of vine may be interpreted as an ovipositional preference.

Initially, only ten females, each three- to six-days old, comprised each experimental trial. But it soon became apparent that neither the number of females nor their age affected ovipositional choice behavior. Each test thereafter involved a minimum of ten females, each at least three days old. A total of fifty females was tested in at least three replicates of each choice situation. No female encountered the same choice more than once. The butterflies were allowed to feed on 1 M sucrose throughout the experiment. Males were not excluded.

Suitability of Passiflora as a food plant

In addition to larval feeding or adult ovipositional preferences, a potentially significant factor affecting the infestation levels of various species of *Passiflora* is the suitability of a particular plant species in terms of the insect's growth and development. In the most extreme cases adult females of several species of insect (including two heliconians—Alexander, 1961b; Benson *et al.*, 1975) have been observed to oviposit on plants toxic to the larvae or on which the larvae will not feed (Straatman, 1962; Sevastopuló, 1964). But toxins, feeding inhibitors, or nutritional imbalances need not be lethal to affect the host-plant range of an insect species. Sublethal toxins and nutritional deficiencies may reduce insect infestation of a particular plant by increasing larval mortality, lengthening larval life, increasing the number of dwarfed adults (which, in butterflies, may reduce mating success), and decreasing fecundity.

These parameters were measured in specimens of *Agraulis* raised on the various local species of *Passiflora* to determine their influence on observed patterns of host-plant infestation.

Larvae, previously unexposed to passion-vine, were collected as they hatched from eggs laid on the nylon screen of the cages by females that had been raised as larvae on either CR, or SP or, in the second experiment, on MN. Thirty first-instar larvae were placed in each of six 5-gallon plastic buckets which contained fresh new-growth shoots of one of the six species of *Passiflora*. Each container

was covered with mosquito netting which prevented the larvae from escaping. The food plant material stood in 50 ml flasks of water and was replaced with fresh vine every other day to avoid excessive deterioration of the plant or larval food shortages. The same specimen of vine provided all leaf material for any one culture throughout all phases of the experiment. As larvae reached the third instar each culture was divided equally between two containers to alleviate overcrowding.

The larvae pupated in their containers. On emergence the adults were transferred to the nylon-screen cages, fed 1 M sucrose from sponges, and the females were allowed to oviposit on fresh new-growth sprigs of AC vine (the species most preferred for oviposition). These cultures were maintained in the laboratory under the conditions outlined previously.

The following parameters were measured: larval and pupal mortality, length of larval life (time, in days, from hatching to 50% pupation), rate of frass production (frass collected, dried, and weighed every other day), adult size (longest dimension of a forewing), and the number of eggs laid by the females. Each of these parameters has previously been used to indicate the suitability of a phytophagous insect's diet (Dethier, 1954; Waldbauer, 1962; Latheef and Harcourt, 1972; Wiklund, 1973; Erickson and Feeney, 1974).

Field survey of Passiflora infestation

A survey of passion-vines in the field was undertaken to evaluate the relative contribution of larval feeding preferences, adult ovipositional preferences, and host-specific patterns of mortality to the distribution of *Agraulis* over various species of *Passiflora*.

The survey took place during the summer of 1974 and involved 45 passion-vines (representing 6 species) located in the cities of Goleta and Santa Barbara, California. Each vine was periodically surveyed by estimating its volume and counting all the specimens of the various life history stages of *Agraulis* found on it. Except for a few vines sampled in May, sampling proceeded once every three weeks from June through August. Thus, each specimen of vine received three to four visits during the peak flight season.

RESULTS

Larval food choice experiments

In the control experiments, fourth-instar larvae consistently deposited more fecal pellets on the agar quadrants labeled with leaves of CR than on the unlabeled quadrants (totals for all ten trials of 454 and 139, respectively; $P < 0.001$; Student's *t*-test). These data correlated well with subjective evaluations of the amount of feeding in each pair of quadrants. Since the larvae voided residual frass prior to the experiment, a significant difference in the number of fecal pellets on quadrants with different labels is interpreted as a feeding choice. The number of pellets on the unlabeled agar is interpreted to be primarily the result of deposition by larvae that wandered after feeding.

Results of the food choice experiments for larvae from each of the five cultures

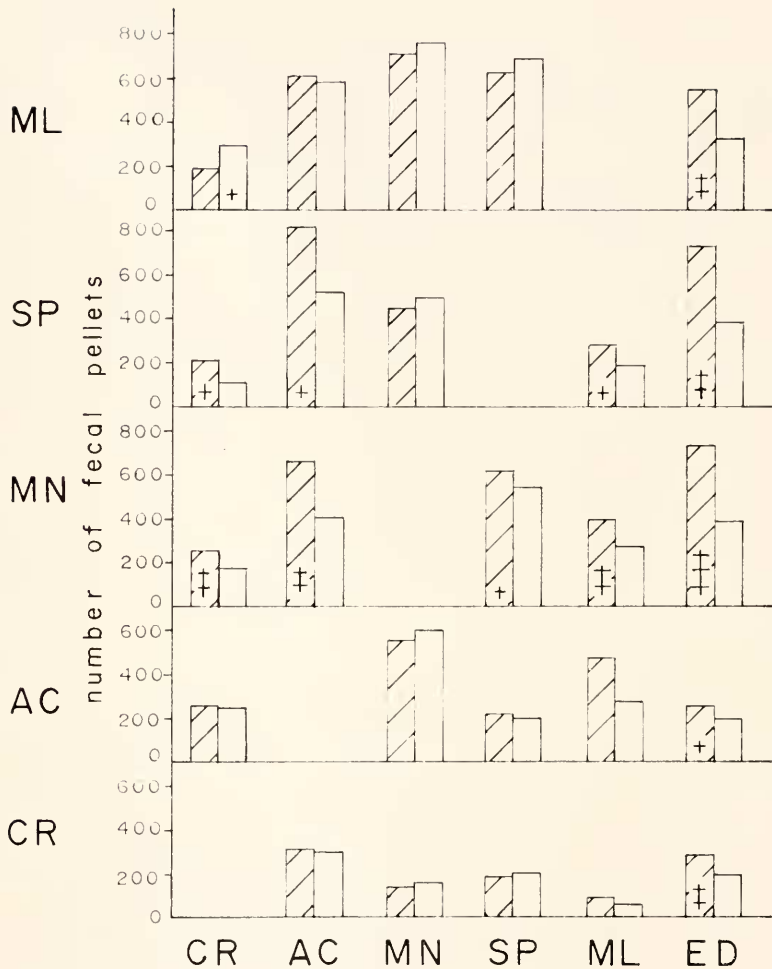


FIGURE 1. Results of larval food choice experiments. Shaded bars indicate number of fecal pellets on agar labeled with host-plant; and open bars, the number of fecal pellets on agar labeled with test plants. Host-plant species appear on the ordinate and test plants on the abscissa. The crosses indicate a significant difference between members of a pair of bars calculated by Student's *t*-test for paired comparisons: +, $0.05 > P > 0.01$; ++, $0.01 > P > 0.001$; +++, $P > 0.001$. Data are not available for ED since larvae did not survive to the fourth instar on that species. CR represents *Passiflora caerulea*; AC, *P. alato-caerulea*; MN, *P. manicata*; SP, *P. sp.*; ML, *P. mollissima*; and ED, *P. edulis*.

are presented in Figure 1. Data for an ED culture are not available, since feeding was minimal and most larvae failed to survive to the fourth-instar on that species of vine.

Comparing, in Figure 1, the choice situations MN *vs.* AC and MN *vs.* CR with CR *vs.* MN and AC *vs.* MN indicates that larvae raised on MN became induced to prefer that species by the fourth-instar. Similarly, larvae reared on

SP became induced to prefer SP over AC and CR. Larvae raised either on AC or CR showed no such induced preferences. Their choices against ED may indicate an avoidance of ED rather than an induced preference for their respective hosts. It should be noted that all larvae chose their host-plant over ED.

These results suggest that MN and SP differ chemically from CR and AC, such that only larvae raised on MN or SP become induced to prefer their host-plants. Two hypotheses explaining this phenomenon are: first, leaves of MN and SP vines contain some compound(s) which effects induction of host preferences in larvae fed these species; and secondly, leaves of CR and AC vine contain an inhibitory factor to which larvae reared on CR or AC become habituated, but to which MN- and SP-raised larvae remain sensitive. The results of preliminary experiments designed to test these hypotheses were inconclusive but tended to support an intermediate interpretation: that CR leaves contained a feeding inhibitor to

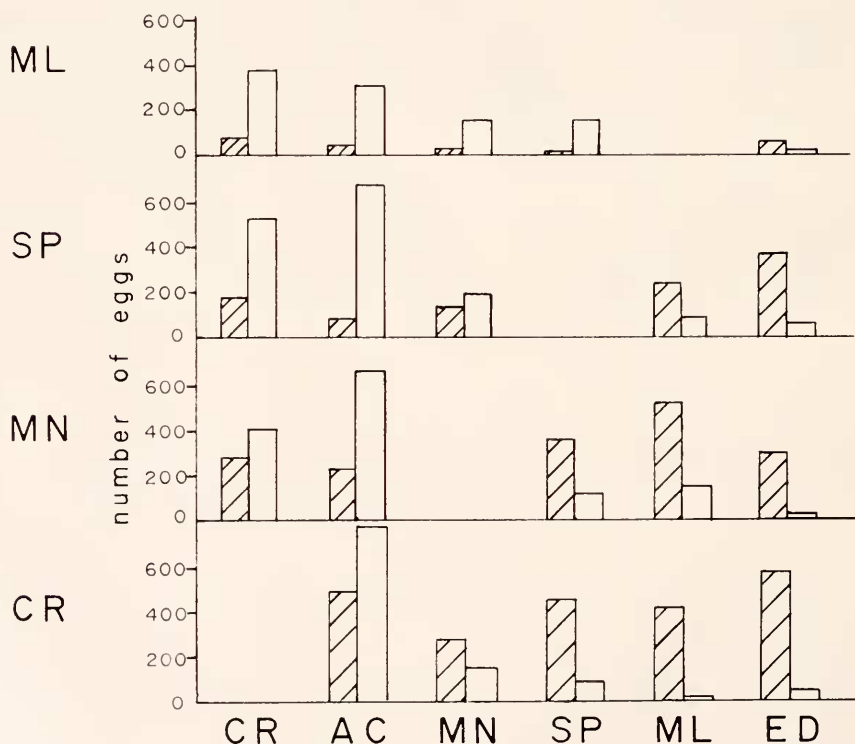


FIGURE 2. Results of adult ovipositional choice experiments. Host-plant species appear on the ordinate, test plants on the abscissa. Shaded bars indicate the number of eggs deposited on the host-plant; open bars, the number of eggs deposited on the test plant. No data are available for the ED and AC host-plants, because of high mortality in butterflies raised on those species. The members of each pair of bars are significantly different from each other as calculated by chi-square, test for goodness-of-fit ($P < 0.001$). CR represents *Passiflora caerulea*; AC, *P. alato-caerulea*; MN, *P. manicata*; SP, *P. sp.*; ML, *P. mollissima*; and ED, *P. edulis*.

TABLE I

Number of eggs laid by *Agraulis* females in ovipositional choice experiments (see Figure 1) involving their host-plant species and one of the other five species of *Passiflora*. Each number represents the average mean value for the number of eggs laid per female in each experimental replicate. Each experiment involved fifty females in at least three replicates, each 48 hr long. The standard error was calculated for the replicates within each experiment. The values for tests involving AC and/or CR in each culture are larger than the next greatest value in that culture (not AC or CR) as indicated by analysis of variance ($P < 0.05$) and tests for least significant difference. AC represents *P. alato-caerulea*; CR, *P. caerulea*; MN, *P. manicata*; SP, *P. sp.*; ML, *P. mollissima*; and ED, *P. edulis*.

Adults raised as larvae on	Mean number of eggs, female in tests involving					
	AC	CR	MN	SP	ML	ED
CR	26.09 \pm 9.15	—	9.08 \pm 4.7	11.47 \pm 12.3	9.1 \pm 5.17	12.98 \pm 7.04
MN	18.81 \pm 4.13	14.62 \pm 2.63	—	8.65 \pm 3.29	13.69 \pm 2.43	7.13 \pm 5.65
SP	16.05 \pm 7.76	13.76 \pm 4.15	7.18 \pm 1.76	—	6.31 \pm 0.15	8.69 \pm 2.97
ML	7.14 \pm 2.14	9.69 \pm 6.15	3.45 \pm 0.93	3.61 \pm 2.3	—	1.62 \pm 0.56

which both CR- and SP-raised larvae were sensitive, and that SP contained some compound(s) that accelerated feeding only in larvae raised on SP (Copp, 1976).

Adult ovipositional choice experiments

In contrast to feeding larvae, ovipositing females made highly significant choices in all experimental situations (Fig. 2). These choices did not vary with larval food plant or food preference. Cross-matching the results yields an ovipositional preference "spectrum" in which AC is the species most preferred, regardless of larval food plant, followed by CR, MN, SP, and ED in that order. Sufficient data to determine preferences were lacking for the AC culture because of high larval mortality and the very low rate of oviposition by the few emergent females.

The ovipositional choices exhibited by females in the laboratory may not be simply relative preferences. Comparing the mean number of eggs deposited per female in each choice situation (Table I) reveals that for any single culture the mean value is significantly greater in those tests with either AC (the tests for the CR, SP, and MN cultures) or CR (the ML culture tests) as one of the choices than in tests with any of the other four species. Furthermore very few eggs were deposited in the ML vs. ED situation. These observations suggest that the ovipositional preferences for AC and CR may result in part from the tendency of females to oviposit at a greater rate in the presence of those vine species, and that the low position of ML and ED in the preference spectrum is due to their apparent lack of any such stimulatory effect. In the field, then, where ovipositing females are presumably not often faced with a single choice situation between two species of *Passiflora* within a few feet of each other, AC and CR would still be more oviposited upon than the other four species investigated, and ML and ED would be oviposited upon very little.

Three factors contributed to some distortion in the ovipositional choice experiments: first, females were confined in a small space with vine, such that, barring very large inhibitory effects, the less preferred vine species probably received

TABLE II

Record of growth and pupation of *Agraulis* larvae raised exclusively on one of six species of *Passiflora*. The data represent the combined totals of two experiments. P50 is the time in days between hatching and formation of 50% of the chrysalids, and E50 is the time in days between P50 and the emergence of 50% of the adults. Total % M is the percentage of larval and pupal mortality. FI is the fecal index (g frass deposited per larva per day). The standard error was calculated for the two experiments. CR represents *P. caerulea*; AC, *P. alato-caerulea*; MN, *P. manicata*; SP, *P. sp.*; ML, *P. mollissima*; and ED, *P. edulis*.

Species of <i>Passiflora</i>	No. of larvae	No. of chrysalids	P50 (Days)	Number Emerged	E50 (Days)	Total % M	FI
AC	60	20	21.3 \pm 0.4	15	7.3 \pm 0.4	75.0	0.023 \pm 0.003
CR	60	55	12.3 \pm 0.3	50	7.5 \pm 0.7	16.7	0.058 \pm 0.014
MN	60	54	11.8 \pm 0.3	42	7.5 \pm 0.2	30.0	0.052 \pm 0.008
SP	60	54	11.8 \pm 0.9	51	8.0 \pm 0.3	15.0	0.051 \pm 0.001
ML	60	32	17.0 \pm 1.4	25	8.3 \pm 1.1	58.3	0.039 \pm 0.010
ED	60	1	25.0	1	8.5	98.3	0.018

more eggs than would be expected under field conditions; secondly, as a result of the close proximity of the vine species during a test (approx. 25 cm) a female, after landing on a specimen of one species of vine which stimulates oviposition, may have moved occasionally to the other vine species before ovipositing; and thirdly, some oviposition occurred in cages devoid of passion-vine. This phenomenon may result from inadvertant selection for such behavior in laboratory cultures of insects (Wearing, Connor and Ambler, 1973). These three factors may have biased the data in favor of the less preferred vine species but are not considered significant enough to interfere with the overall interpretations.

Suitability of Passiflora as a food plant

This survey was conducted first from May to June, 1975, and repeated from January to February, 1976. Since the results of the second survey did not differ significantly from the results of the first, the two sets of results are combined and presented together (Tables II and III).

Larvae fed on CR, SP, and MN grew to pupation at more or less equally high rates followed by ML-, AC-, and ED-raised larvae, in that order. Mortality of larvae and pupae followed a similar pattern across the vine species; it was lowest in the CR and SP cultures and progressively higher in the MN, ML, AC, and ED cultures.

The low rate of larval maturation and high larval and pupal mortality evident in the AC, ML, and ED cultures correlate with low rates of larval frass production in these cultures (Table II). Since the rate at which frass is produced may indicate the rate at which food is consumed (Mathavan and Pandian, 1974), these results suggest that leaves of AC, ML, and ED possess factors which inhibit feeding by larval specimens of *Agraulis*. However, the possibility that the differences in the rate of frass production among the various cultures resulted from differing percentages of food utilization cannot be excluded without further experimentation.

The emergent adults in each culture were compared by measuring the length

of a forewing. In some other butterfly species it has been shown that wing length is correlated with the amount of food assimilated during larval instars, and that those adults with larger wings tend to enjoy greater mating success (Wiklund, 1973). Mean wing lengths for both males and females were about equally large in the CR, MN, and SP cultures (Table III) and smaller in the other three cultures. Females were slightly, but significantly, larger than males in the CR and SP cultures and slightly smaller than males in the ML culture. No sexual dimorphism in wing length appeared in the other three cultures. One very small female emerged in the ED culture. It was mated with a male from the CR culture but died before ovipositing.

Unexpectedly, the number of eggs laid by females in each culture did not consistently follow predictions based on adult size (Table III). Unlike butterflies in the genus *Heliconius* which extract amino acids from pollen (Gilbert, 1972), *Agraulis* has no external source of amino acids aside from those present in nectar (Baker and Baker, 1973; Benson, Universidade Federal do Rio de Janeiro, personal communication). Butterflies such as *Agraulis* must depend on reserves stored during larval instars for proteinaceous material used in egg production (Engleman, 1970). Consequently, low rates of larval feeding should result in small females that lack sufficient protein reserves for extensive egg production. Females from the AC culture were among the smallest produced and, indeed, they laid relatively few eggs ($AC < CR$, $P < 0.001$; $AC < ML$, MN , and SP , $P < 0.01$; chi-squared test for goodness-of-fit; Table III). Contrary to predictions, the small females from the ML culture deposited about as many eggs as the larger females from the MN and SP cultures. Possibly, females in the ML culture had assimilated sufficient protein reserves for oviposition as a result of feeding as larvae for a longer period of time, albeit at a lower rate, than larvae in the MN and SP cultures.

In general, for each of the parameters measured, *Agraulis* developed and reproduced as well or better on CR as on any of the other five species of

TABLE III

Record of adult Agraulis raised as larvae exclusively on one species of Passiflora. These data represent the combined totals of two experiments. The longest dimension of a forewing was taken as a measure of adult size. P indicates probability that there is a significant difference between the mean size of females and males from a single culture (calculated by Student's t-test; $P > 0.05$; n.s., not significant). The standard error for the mean number of eggs was calculated for the two replicate experiments. CR represents P. caerulea; AC, P. alato-caerulea; MN, P. manicata; SP, P. sp.; ML, P. mollissima; and ED, P. edulis.

Adults raised as larvae on	Number of adults	Wing length (mm)		P	Mean number of eggs laid per female
		Males	Females		
AC	15	34.2 \pm 2.9	34.3 \pm 3.8	n.s.	161.1 \pm 69.3
CR	50	35.7 \pm 2.4	38.1 \pm 2.4	$P < 0.001$	300.0 \pm 66.2
MN	42	36.8 \pm 1.4	37.8 \pm 1.7	n.s.	262.6 \pm 51.6
SP	51	36.9 \pm 2.6	38.7 \pm 2.3	$P < 0.05$	232.6 \pm 65.9
ML	25	34.9 \pm 2.3	33.6 \pm 1.8	$P < 0.05$	248.8 \pm 6.7
ED	1	(none)	32.0		0

TABLE IV

Results of the survey of *Passiflora* infestation by *Agraulis*. Each vine was sampled once every three weeks for three months during the summer of 1974. At each sampling the number of *Agraulis* eggs, larvae, pupae and adults was counted. The infestation level (mean number of *Agraulis* per m^2) for AC is significantly greater than for the other vines ($P < 0.05$; Student's *t*-test). AC represents *P. alato-caerulea*; CR, *P. caerulea*; MN, *P. manicata*; SP, *P. sp.*; ML, *P. mollissima*; and ED, *P. edulis*.

Species of <i>Passiflora</i>	Number of vines sampled	Mean size of vine (m^2)	Mean number of <i>Agraulis</i> / m^2
AC	28	2.66 ± 4.34	53.05 ± 32.1
CR	7	3.0 ± 2.91	10.84 ± 8.51
MN	5	3.5 ± 2.54	13.85 ± 12.34
SP	1	1.36	5.15
ML	2	13.6 ± 9.62	0
ED	2	9.64 ± 2.88	0

Passiflora tested. Larvae also grew and developed well on MN and SP, but larval mortality was higher in the MN culture and females laid fewer eggs in the SP culture than in the CR culture ($P < 0.001$, chi-squared test for goodness-of-fit). Larvae fed on ML, AC, and ED grew at a reduced rate with high mortality and produced small adults.

Field survey of *Passiflora* infestation

Based on the foregoing experimental results, it was predicted that CR would be the most infested species because it is highly preferred for oviposition and highly suitable for the growth and development of *Agraulis*. According to these indices, MN and SP should be the second most infested (both support larval development but neither is as preferred for oviposition as CR) followed by AC (highly preferred for oviposition but poor for growth and development). The remaining two species, ED and ML, should not become very infested in the field since few eggs are deposited on either in the laboratory.

These predicted relative levels of infestation were arrived at by considering that the larval feeding behavior would have no direct effect on host selection (passion-vines are generally too widely spaced for successful larval immigration; personal observation), and that the effects of larval mortality and adult oviposition would be of approximately equal importance and either balance or complement each other. The results suggest that this is not the case (Table IV). The infestation levels follow closely a pattern that would be predicted on the basis of ovipositional preferences alone, with the exception that CR did not become more infested than MN.

Considering the unequal number of specimens available for each species of vine, and possibilities of local variations in parasitic load on *Agraulis*, inferences based on these results concerning the relation of larval and adult influences on host selection must remain tentative at best. However, in the case of ML it is likely that its failure to stimulate a high level of oviposition is responsible for the total lack of infestation by *Agraulis* since both ML vines sampled grew within a few meters of other, heavily infested passion-vines, and ML can support larval growth.

DISCUSSION

The experimental results reported here indicate that *Agraulis vanillae incarnata* (Riley) in Santa Barbara infests its host-plants in the genus *Passiflora* primarily to the degree that each species of vine stimulates oviposition by the female butterflies. However, other, untested factors may influence patterns of host-plant infestation by *Agraulis*, such as the geographical distribution of adult butterfly nectar resources (Gilbert and Singer, 1973) and butterfly predators.

Larval feeding behavior plays a very minor role, at best, in host-plant selection by *Agraulis*. Larval immigration between host-plants is considered highly unlikely given the usually great distances between passion-vines. Consequently, larval feeding preferences could not directly affect host-plant selection (Dethier, 1959). However, induced larval feeding preferences may affect the infestation level of a particular host-plant if such preferences reduce the rate at which larvae wander from the plant and perish. This may explain why MN became as infested as CR even though the former species is less preferred for oviposition.

The feeding experiences of *Agraulis* larvae did not indirectly affect host-plant selection by influencing the ovipositional preferences of the subsequent females as predicted by Hopkins' host selection principle. Given that adaptation to a host-plant species entails a decreased capacity on the part of the insect to grow and develop on a variety of other nonhost species (Gordon, 1961), it is difficult to imagine that the mechanism of Hopkins' host selection principle, whereby larval feeding experience is transferred to the ovipositional preference of subsequent adult females, would be selected for in any but the most polyphagous species of insect.

Rather than invoke Hopkins' host selection principle to explain the origin of specific insect-plant associations, it seems reasonable to suppose that adult ovipositional preferences are genetically determined and evolve in accordance with the survival of the larvae on various potential host-plant species, as proposed for *Papilio machaon* by Wiklund (1975). *Agraulis* females would thus be expected to oviposit preferentially on those species of *Passiflora* most conducive to larval growth and development. In a broad sense, this expectation was realized but with a few notable exceptions. Two of the three most preferred species for oviposition, CR and MN, were also highly suitable as larval food plants, and the two species least preferred for oviposition, ML and ED, were among those least favorable for larval growth. In contrast, AC was the most preferred species for oviposition in the laboratory and the most infested species in the field, yet the indicators of host-plant suitability suggest that AC is one of the least suitable food plants for *Agraulis* larvae. A possible explanation for this disparity is that larvae in the field may feed on older, possibly less toxic, foliage than they were provided in the laboratory. In this way the larvae of *Euclides isabella* avoid toxins in young leaves of *P. serratifolia* (Benson *et al.*, 1975). Alternatively, if, as suspected, the ovipositional preference for AC observed in the laboratory has an absolute significance in the field, then more eggs would be deposited on that species of vine than on any other, possibly enough to compensate for a high rate of mortality.

Another inconsistency with the above prediction is that ML and SP, each

apparently more suitable as a larval food plant than AC, were so little oviposited upon. The explanation may be found in the context of the ongoing reciprocal interactions intrinsic in coevolutionary relationships: ML and SP may have temporarily "escaped" heavy *Agraulis* predation pressure by virtue of their lack of oviposition stimulants.

Alternatively, these inconsistencies may be interpreted as evidence that a more static relationship is still in the process of formation. This is a distinct possibility, since passion-vines were not imported into Santa Barbara County until about 1900. *Agraulis*, presumably, was either transported along with the vine or followed in a gradual northward extension of its range. Considering the relatively short generation time of *Agraulis* (approx. 1 month under laboratory conditions), considerable adaptation to the local flora could have occurred in the past eighty years. No doubt this is the case, since the only local species of *Passiflora* that occurs in the records of host-plants for *Agraulis* in the tropics is *P. edulis* (Benson *et al.*, 1975), a species not infested locally. However, the local species of *Passiflora* probably do not accurately reflect adaptive responses to predation by *Agraulis*, since they are subject to extensive human manipulation.

In spite of these qualifications, the important role of the ovipositional preferences of *Agraulis* in determining levels of *Passiflora* infestation is probably a basic element of their association that has been carried over from its tropical origin.

We believe that, although this somewhat artificial system has not provided great insight into the course of heliconian-*Passiflora* coevolution in tropical America, its apparent simplicity has offered us a unique opportunity to investigate the behavioral control of such an association isolated from the complexities of tropical ecosystems.

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SUMMARY

1. Prior to an investigation of the sensory modalities and behavior involved in host-plant selection by *Agraulis vanillae incarnata* (Riley), experiments were conducted to evaluate the relative influence of larval feeding behavior, adult ovipositional behavior, and host-specific butterfly mortality in determining the infestation of various species of host-plant in the genus *Passiflora*.

2. The larvae demonstrated induced feeding preferences for some, but not all, species of *Passiflora* upon which they had previously been raised.

3. The adult females exhibited definite, fixed ovipositional preferences among six species of *Passiflora* available in Santa Barbara County. These preferences were not affected by either larval diet or induced feeding preference.

4. The food plant suitability of the six local species of *Passiflora* varied con-

siderably as indicated by several aspects of *Agraulis* growth, development, and reproduction. With a few notable exceptions this food plant suitability broadly correlated with the adult ovipositional preferences as predicted by Wiklund's model for the evolution of ovipositional preferences. The exceptions are discussed in terms of what they may reveal about the development of the *Agraulis*-*Passiflora* association in southern California.

5. The results of a field survey of host-plant infestation by *Agraulis* did not entirely fit predictions based on the above experimental results. The levels of infestation of the various species of *Passiflora* appear to correlate more closely with the ovipositional preferences of the adult females than with any of the other parameters studied.

6. The ovipositing females primarily determine the degree to which each species of *Passiflora* in Santa Barbara County becomes infested. The influence of larval behavior is negligible. The distribution of *Agraulis* among its host-plants is the result of an active process and is not, except in an evolutionary sense, determined by passive factors such as mortality on unsuitable food plants.

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